Microgeographic variation in morphology within populations of threespine stickleback

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Abstract

Local adaptation, the evolution of traits that make native individuals in a population have higher fitness than foreign individuals, is studied intensively in the field of evolutionary biology. For local adaptation to occur, different habitats must select for different traits, and this divergent selection must be stronger than the homogenizing effect of selection. However, at small spatial scales, gene flow is believed to be the stronger evolutionary force, eroding any local adaptation. Yet, local adaptation can still occur at small spatial scales ('microgeographic divergence') if selection is strong, if individuals proactively choose their habitat, or if philopatric individuals adjust their phenotypes to their local environment. The goal of my research is to test for microgeographic divergence within populations of threespine stickleback. I analyzed morphological and trap data of threespine stickleback collected in 2013, to test for among-trap differences in morphological traits. I show that stickleback morphology differs among traps within lakes, and within streams. This microgeographic variation is stronger for some traits than for others and is only partly attributable to isolation by distance within lakes. Overall, the results provide support of microgeographic divergence, a concept that may be more widespread than evolutionary biologists have previously believed.

Introduction

The idea of local adaptation is a well-known concept in evolutionary biology. However, local adaptation at small spatial scales (microgeographic adaptation) is not widely researched. This is mainly due to the belief that high gene flow, the exchange of genes from one population to another, may prevent local adaptation from occurring at small spatial scales (Richardson, Urban, Bolnick & Skelly, 2014). These small spatial scales can be defined as within an organism's dispersal neighborhood (Sewall Wright's dispersal neighborhood), which is the area where dispersal is frequent enough to prevent genetic drift (Richardson, Urban, Bolnick & Skelly, 2014), and can be expected to entail high gene flow. 'Dispersal' is used to define the displacement of offspring away from parents. Microgeographic divergence differs from microgeographic adaptation, in that microgeographic divergence does not make any assumptions about the adaptive value about the variation in traits, or about any genetic basis to this variation. My research seeks to identify whether there is microgeographic divergence in threespine stickleback within populations and explore the possible mechanisms facilitating it, such as assertive schooling, microhabitat choice, and phenotypic plasticity.

Migration-selection balance describes the relationship between gene flow and divergent selective pressures (Graham et. al, 2017). Divergent selective pressures arise from variations in the environment. However, local adaptation and continued divergence depends on the balance between the strength of selection and gene flow in relation to each other (Nosil et al., 2009). When selection is stronger relative to gene flow, divergent selection can be experienced. When gene flow is stronger than selection, we expect to see homogenization of traits across the landscape (Graham et al., 2017). However, at small spatial scales gene flow is typically stronger

than the strength of selection, facilitating homogenization. Therefore, biologists typically do not expect microgeographic divergence.

Despite this theoretical expectation, examples of microgeographic adaptation are known in different organisms, such as the peppered-moths (*Biston betularia*), land snails (*Cepaea nemoralis*), and grass (*Anthoxanthum odoratum)* (Richardson, Urban, Bolnick & Skelly, 2014; Richardson & Urban, 2013). The peppered-moth (*Biston betularia)* in England and its coloration before and after industrialization is a well-known example of microgeographic adaptation with a selective barrier in nature. These moths have a dispersal neighborhood of about 54-km (Saccheri, I.J. et al., 2008). Its population was dominated by the light-colored moths before industrialization spread throughout England, allowing for an advantage over darker-colored moths due to its ability to camouflage in the lichen-colored trees. However, these lichen-covered trees soon became soot-covered as industrialization spread, causing the darker phenotype of the moth to become more prominent and to be at a greater advantage for camouflage and avoiding visual predators. Research has shown that selective barriers created by visual predators caused microgeographic adaptations in the peppered moth, in the spatial scale of about 10-km. Over time the pollution decreased, and the colors of the moths mirrored the change in air quality and showed differences in color. However, with such a broad dispersal neighborhood, these moths should have experienced gene flow that would prevent the domination of light and dark morphs, indicating that selection was stronger than gene flow (Saccheri et.al, 2008).

Another example illustrating microgeographic adaptation is divergence of metal tolerance and grass flowering phenology of *Anthoxanthum odoratum* (Antovonics, 2006). This divergence occurred spanning an area less than 20 m, a small spatial scale considering this species of grass is

wind pollinating and therefore pollen dispersal should create gene flow that prevents short clines of metal tolerance and flowering phenology.

While examples of microgeographic variation exist, the concept is still often overlooked because migration-selection balance theory tells us not to expect such variation within dispersal neighborhoods. But, classical migration-selection balance theory omits several biological processes that can generate divergence despite, or because of, high gene flow. First, habitat choice and genotype dependent dispersal can facilitate fine-scale population differentiation despite gene flow (Bolnick & Otto, 2013). While it is often assumed that migrants carry a random sample of regional alleles (Lenormand, 2002), there is evidence that genotypes can differ in dispersal ability or habitat preference (Edelaar, Siepielski & Colbert, 2008). The nonrandom distribution of these genotypes in space can promote microgeographic adaptation if the spatial segregation involves matching phenotypes to habitats conferring higher fitness (Bolnick & Otto, 2013). Habitat choice has been found in stickleback preferentially dispersing into lake or stream habitats, and therefore promoting their divergence in a small spatial scale of a few meters upstream from a lake (despite the capability of stickleback to disperse hundreds of meters in a few days) (Bolnick et al., 2009). Second, phenotypic plasticity can also promote microgeographic variation if individuals are territorial and adjust their traits to local environments. For example, phenotypic plasticity in nuptial color of threespine stickleback has been found as the primary mechanism involved in producing a depth gradient in conspicuousness over a small spatial scale (<2 meters) (Brock, Cummings & Bolnick, 2017). Third, assortative schooling can serve as source of phenotypic variation among groups that will look like microgeographic variation but isn't anchored in space. Assortative schooling is self-referent matching behavior exhibited in social animals with regard to phenotype and preferentially

matching with conspecifics (Ward, Hart & Krause, 2004), and instances of assortative schooling have been found in threespine stickleback developing preferences for unrelated familiar individuals (Utne-Palm and Hart, 2000). My research seeks to answer whether microgeographic divergence exists within populations of threespine stickleback.

Materials and Methods

Study System

The threespine stickleback, *Gasterosteus aculeatus,* is a small fish usually between 3cm-8cm long that maintains a large amount of morphological variation. We analyzed the morphologies of stickleback that inhabit stream environments, and lake environments, on Vancouver Island in British Columbia. Studies of stickleback have documented trait divergence between populations in these two habitats (Stuart et al 2017). Lake stickleback tend to have longer and a greater number of gill rakers than stream stickleback in order to feed on zooplankton. Lake stickleback also have smaller streamlined body to aid in sustained swimming compared to the larger, deeper body of a stream stickleback (Bell, 1982; Berner et al., 2009; Kaeuffer et al., 2012). Studies have also found there is variation of traits within the same habitat. For example, (Izen, Stuart, Jiang & Bolnick, 2016) found there is covariation between fin morphology or gill raker length and local flow regime in different streams and is most likely due to non-random dispersal. Their results provide an example of within-habitat microgeographic variation. (Brock, Cummings & Bolnick, 2017) also found male stickleback conspicuousness (signal design) varies over small spatial scales within the lake habitat depending on the male's nest depth, another example of microgeographic variation within a population. Snowberg et al (2015) found diet covarying with

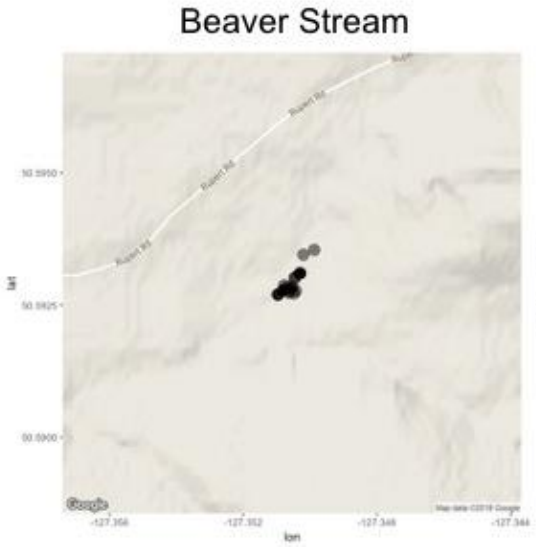
nest depth within a stickleback population. Bolnick et al (2015) found that immune phenotypes covaried with nest depth within lakes as well.

Many phenotypic traits have been associated with ecological adaptations in stickleback. One of the determining factors of feeding behavior is the gill raker, which vary in number, spacing, and length. Gill rakers play a large role in limiting the size of food particles eaten by fish, in that a higher number of gill rakers usually manifests as a diet better suited for filter feeding of organisms such as zooplankton, while a lower number signifies a diet better suited for foraging (Glazer et al., 2014). For protection against predators, stickleback have lateral armour plates, which provides protection against predators. Stickleback possess sharp dorsal and pelvic spines that serve the same defensive purpose. When a larger fish attempts to eat a stickleback, the stickleback extends its spines, deterring the predator (Wiig et al., 2016). Traits that help to determine maneuverability include the pectoral fin, dorsal fin, and caudal fin, with different combinations of sizes determining how well adapted the fish is in moving around in its respective environment (Walker, 2004).

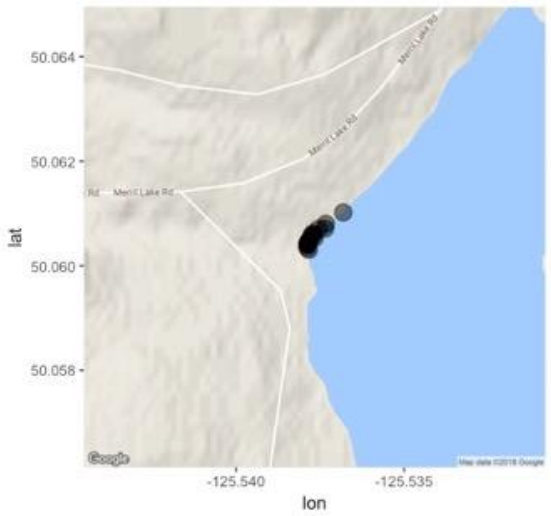
Study design

In 2013, Stuart et al (2017) collected threespine stickleback from one lake, and its corresponding outlet stream, from each of the 16 watersheds on Vancouver Island in British Columbia (32 sites total). They caught fish using fifty unbaited minnow traps, set across a transect of about 100 meters in each of the 32 total sites to include all available habitat at a site, except very deep locations that stickleback rarely use. To visualize the distribution of traps within each site, I plotted the coordinates of each trap **Figure 1)**.

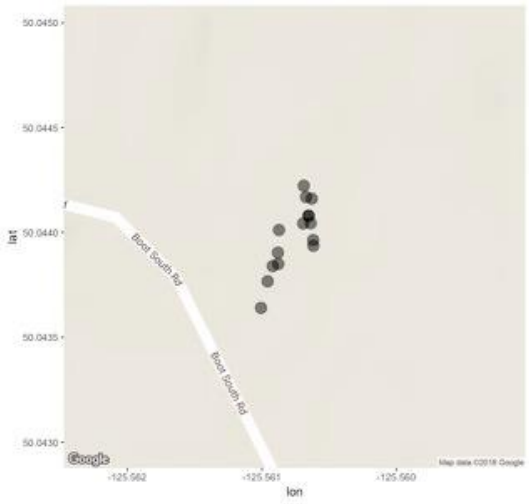
To describe the environment for each habitat, Stuart et al (2017) measured environmental variables for at least 50 traps per site, which included flow rate, depth, substrate, and vegetation. To obtain morphological data, Stuart et al (2017) chose approximately 40 stickleback per site that have been preserved for at least 14 days, and collected linear measurements and body shape information through the use of digital landmarks placed on photographs, and estimated body shape through the use of geometric morphometrics. Details of the environmental and phenotypic measurements can be found in the supplementary materials of Stuart et al (2017).



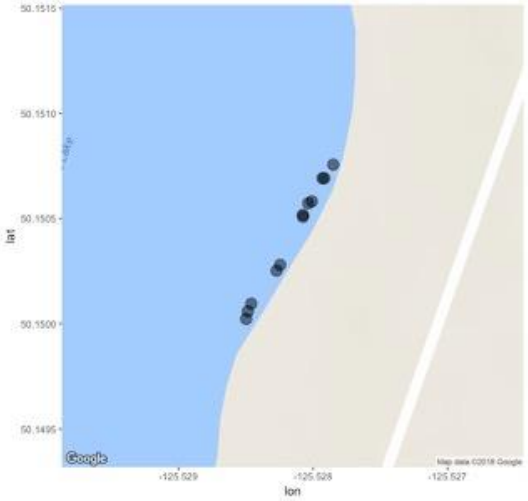
Boot Lake



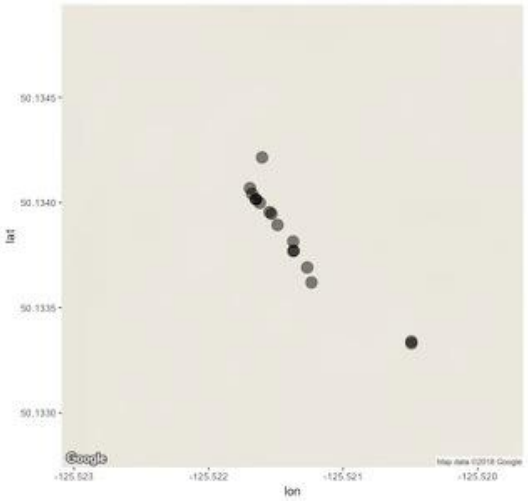
Boot Stream



Comida Lake

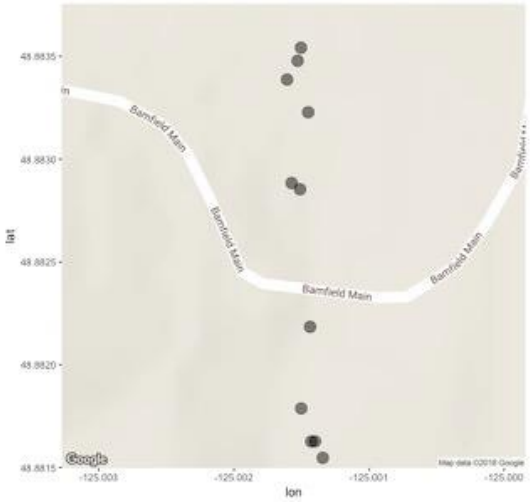


Comida Stream



Fredrick Lake

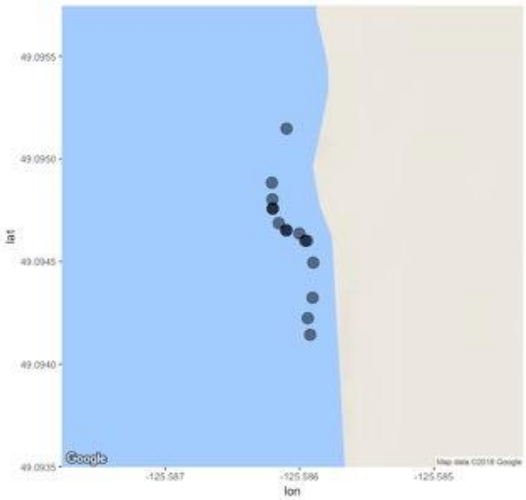
Fredrick Stream



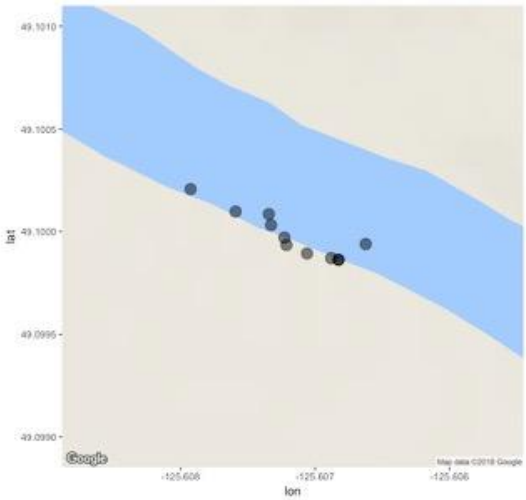
Joe Lake

Joe Stream

Kennedy Lake

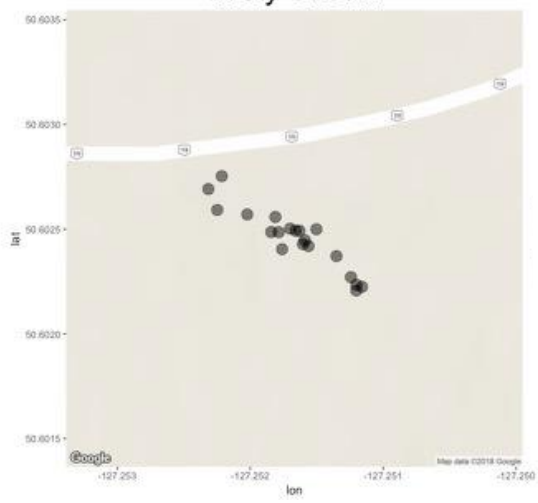


Kennedy Stream



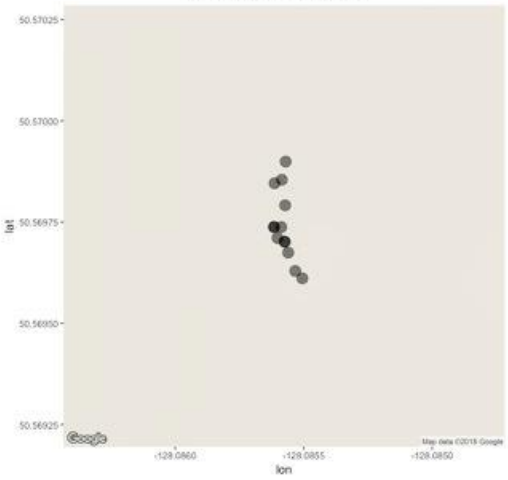
Misty Lake

Misty Stream



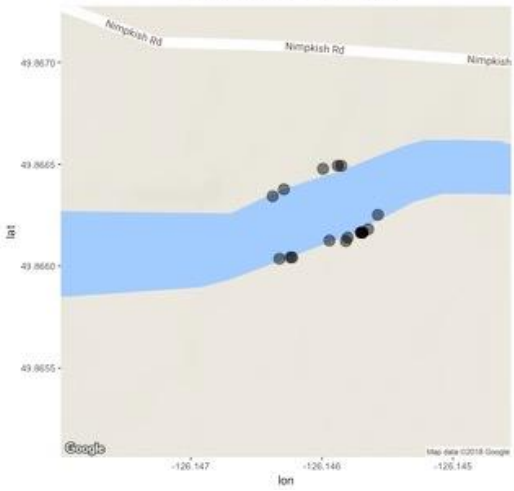
Moore Lake

Moore Stream

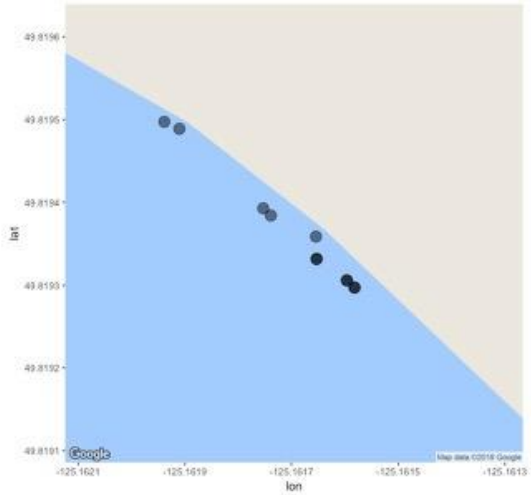


Muchalat Lake

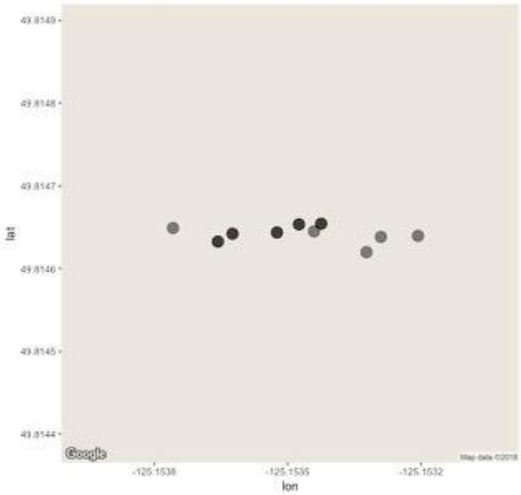
Muchalat Stream



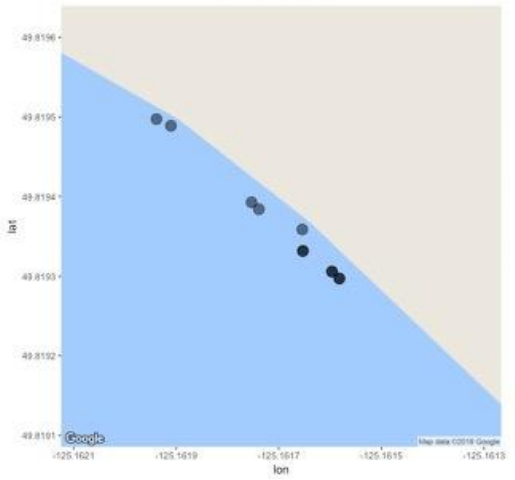
Northy Lake



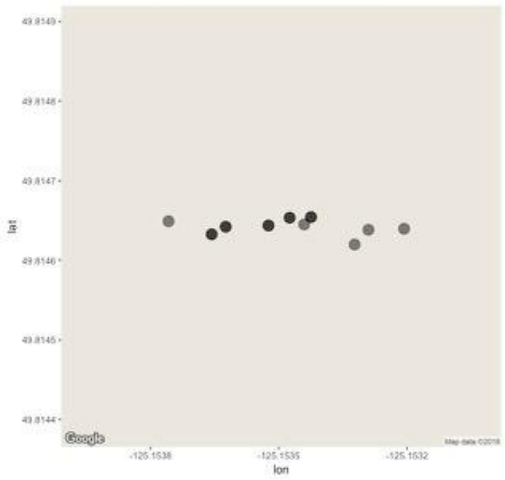
Northy Stream



Northy Lake

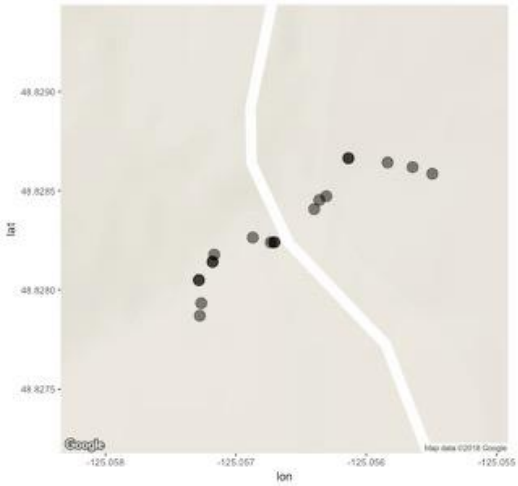


Northy Stream

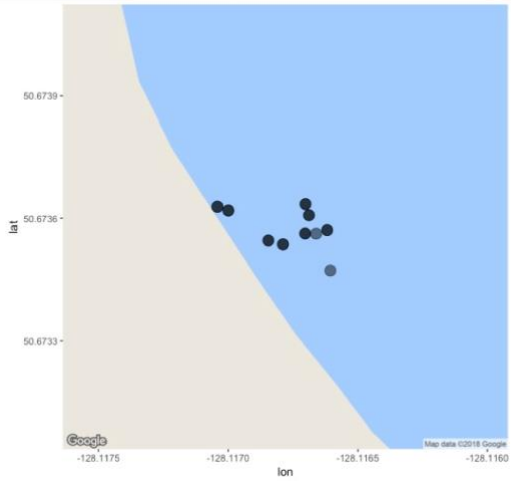


Panchena Lake

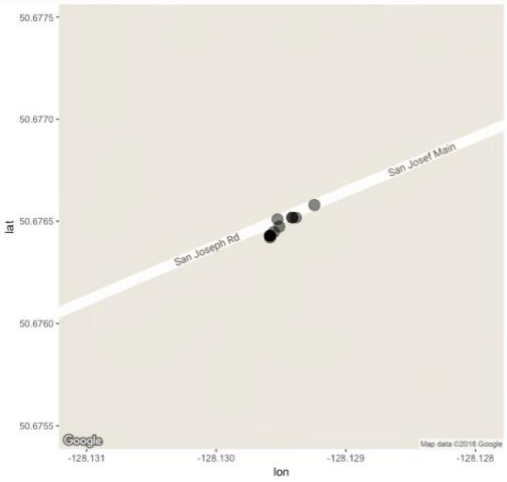
Panchena Stream



Swan Lake



Swan Stream



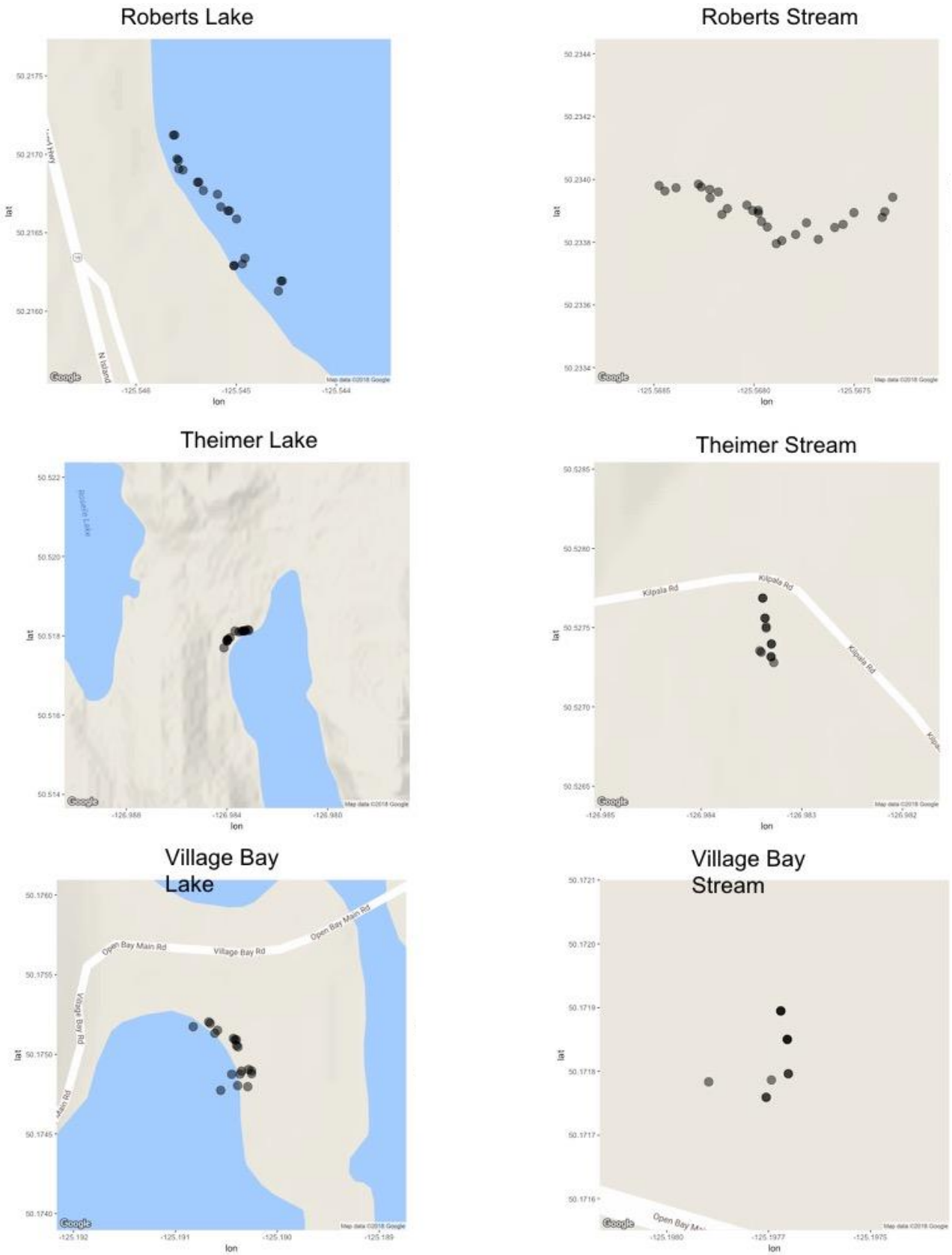


Figure 1. Distribution of traps within each site based on coordinates. Each dot is a trap

location. Maps were drawn using the ggmap package in R. Points were converted from UTM to degrees and plotted using the proj4 package. Slight errors in GPS measurements and GoogleEarth maps' lake boundaries lead to some traps incorrectly appearing to be on land.

Analysis

I tested whether microgeographic divergence existed between traps within populations (individual lake habitat or individual stream habitat) of threespine stickleback, using Bayesian hierarchical linear modeling. I first made size corrections in R-studio for twenty-nine morphological traits using the formula, $M_{s,i} = M_{0,i} \times (L_s/L_{0,i})^b$, where $M_{s,i}$ is the size-corrected trait value for individual *i*, $M_{0,i,}$is the non-size-corrected trait value for individual *i*, L_s is the overall mean for the log-transformed size-related variable across all individuals, and $L_{0,i}$ is the log-transformed size-related variable of individual *I,* and *b* is the common within-group slope (Stuart et al., 2017).

I then used the following statistical model to analyze for among-trap variation in stickleback morphology (total of 39 morphological traits). Our goal was to estimate the magnitude of trap-to-trap variance in any phenotypic trait, Y. I assumed Y was a normally distributed trait (after log transformation and size correction), with mean μ and variance σ:

$Y \sim N(\mu,\sigma)$

Then, I assumed that the expected trait value for a given trap *i* varies around some region-wide trait mean, alpha, for lake fish. Stream fish are assumed to have a different trait mean which is greater or less than the lake expectation by an amount α_{stream}. Likewise, each watershed deviates from this grant mean by an amount $\alpha_{watershed[i]}$**.** Finally, within each population, each trap will differ from the population expectation (α+ $\alpha_{watershed[i]}$+α_{stream}) by a trap-specific amount, $\alpha_{trap[i]}$**.**This can all be written as the following linear equation for the expectation for the trait mean in trap$_{[i]}$ in habitat$_{[j]}$, $\mu_{[ij]}$:

$\mu = \alpha + \alpha_{trap[i]}+\alpha_{watershed[i]}+\alpha_{stream}h_j$

Next, I assumed broad but pre-defined Bayesian prior probabilities for the regional trait mean, and the fixed effect of habitat:

$\alpha \sim N(0,10)$

$\alpha_{stream} \sim N(0,10)$

I used adaptive hyperpriors for the random effects of individual trap and watershed. This means we are using the Bayesian model to estimate the magnitude of among-trap and among-watershed variances:

$\alpha_{trap[i]} \sim N(0, \sigma_{trap})$

$\alpha_{watershed} \sim N(0, \sigma_{watershed})$

These variances were given Cauchy priors:

$\sigma_{trap} \sim Cauchy(0,1)$

$\sigma_{watershed} \sim Cauchy(0,1)$

$\sigma \sim Cauchy(0,1)$

The model gathered 1,000 samples to estimate parameters from the posterior probability distribution using the Hamiltonian Monte Carlo (HMC) algorithm implemented the map2stan package (McElreath, 2016) in R version 1.0.136. and the *rethinking* package (McElreath, 2016). I created a matrix dataset which contained the precis results of the model (**Table 3)** and also created a visual representation of the posterior probability densities of the mean σ_{trap} for each morphology (**Figure 2).**

I calculated Widely Applicable Information Criterion (WAIC) measures of fit for the full model, and compared this to the WAIC of models lacking σ_{trap}. The model that does not contain σ_{trap} is:

$Y \sim N(\mu,\sigma)$

$\mu = \alpha + \alpha_{watershed[i]} + \alpha_{habitat} h_j$

$\alpha \sim N(0,10)$

$\alpha_{habitat} \sim N(0,10)$

$\alpha_{watershed} \sim N(0, \sigma_{watershed})$

$\sigma_{watershed} \sim Cauchy(0,1)$

$\sigma \sim Cauchy(0,1)$

There were three traits in which I used a model that contained a poisson distribution instead of a normal distribution. These traits include left plate count, right plate count, and right side gill raker number (insitu) because these traits are not normally distributed since they are counts, and were not size-adjusted. The model for these traits is:

$Y \sim Poisson(\mu)$

$Log(\mu) = \alpha + \alpha_{trap[i]} + \alpha_{watershed[i]} + \alpha_{habitat} h_i$

$\alpha \sim N(0,10)$

$\alpha_{habitat} \sim N(0,10)$

$\alpha_{trap} \sim N(0, \sigma_{trap})$

$\alpha_{watershed} \sim N(0, \sigma_{watershed})$

$\sigma_{trap} \sim Cauchy(0,1)$

$\sigma_{watershed} \sim Cauchy(0,1)$

I then performed Widely Applicable Information Criterion (WAIC) model comparisons with the WAIC function in the rethinking package with map2stan (McElreath, 2016), comparing the models containing σ_{trap} with models that do not, as well as the pWAIC values. The values of the WAIC are sorted from small to large values, with the small values indicating a better

estimated out-of-sample deviance than a larger value (**Table 1)**. The pWAIC values were also looked at to interpret how convincing the model is, with the higher number representing a more convincing model.

To visualize the distance between traps, I also plotted each trap according to its respective latitude and longitude. In addition, I are able to see if there is clustering of traps along the shoreline of a given habitat. I also analyzed the distances between individual traps by finding the mean distances and standard deviations between adjacent traps (nearest neighbor) within a site, and also overall mean distance and standard deviation of all traps within a site (**Table 2)**. By doing so, I can quantify the spatial scale at which traps were separated from each other within a habitat and are able to look at the ratios between the mean and standard deviations in trap distances. In addition, I calculated the differences in values of morphological traits between traps. To test whether there is a correlation between trait trap-trap distance and trap distance, I performed Mantel tests in R-studio, using the differences in traits between traps and the geographic distance between traps, both within the same site. I hypothesized that spatially distant traps are also morphologically distant, and therefore since Pye Lake has a much higher mean distance between traps compared to standard deviation, I also predict Pye Lake will have correlation between spatial and phenotypic difference due to spatial isolation. I analyzed eight different traits that had the highest mean σ_{trap} value (**Tables 4-11)** The Mantel test gave us a correlation coefficient ("mantelr") and a p-value ("pval1") which allowed us to interpret whether there is a correlation between spatial distance and phenotypic differences. A significant one-tailed p-value less than 0.05 signifies a correlation, where traps that are further apart spatially have fish that are more phenotypically different. However, a p-value greater than 0.05 signifies that the variation we see in traits is apparently not attributable to spatial isolation.

Since it is possible that due to a low statistical power I was not able to see significant spatial isolation by distance in any one habitat, I also looked at all the sites combined and their correlation coefficients. It is also possible that while the correlation coefficients are positive, they were not significant enough for spatial isolation. For the same traits that I performed Mantel tests on, I also created histograms to first visualize the distribution of correlation coefficients and whether it is centered around zero or skewed. I then performed one-sample t-tests with the correlation coefficients to see if they are consistent with an average of zero, and therefore determine if there is an overall trend towards spatial isolation with all sites that I may not be seeing with individual sites.

Results

The results of the analysis showed that some degree of among-trap variation exists for almost all stickleback morphological traits (out of the total thirty-nine that were looked at), with anal fin length having the least amount of variation. Right plate count and left plate count have the strongest aggregation out of all the morphological traits, and their correlation to each other explains the similarity in their distributions. The analysis of WAIC model comparison concludes that the statistical models which includes σ_{trap} are better and more convincing models than ones that do not include σ_{trap}. Overall, thirty morphological traits had a higher WAIC value when their models possessed σ_{trap} compared to the ten that did not. Additionally, thirty-seven traits had a higher pWAIC value when σ_{trap} was included in the model (**Table 1)**.

After calculating the mean distances and standard deviations between adjacent traps (nearest neighbor) within a site, and also overall mean distance and standard deviation of all traps within a site, I found that a much higher mean compared to variance signified clustering in traps, such as Pye Lake where the mean distance between traps is 1032.408 meters while the standard deviation is 795.27 meters. I also ran Mantel tests on eight morphological traits that had the highest mean σ_{trap} values. Each morphological trait had varying correlation coefficients and p-values between individual habitats. The majority of p-values for each morphological trait were greater than 0.05. However, there were certain sites that had a p-value lower than 0.05 for more than one morphological trait. For example, Pye Lake, Misty Stream, Theimer Stream, and Comida Lake all had significant p-values for four (out of the eight) morphological traits that were analyzed. Joe Stream had a significant p-value for three morphological traits, and overall thirteen out of the thirty-two sites had at least one significant p-value, signifying a correlation between spatial and phenotypic difference (**Tables 4-11).** This result is remarkable, because it implies phenotypic isolation-by-distance over spatial scales that stickleback readily traverse within a few days.

After running one-sample t-tests on the each of the same eight morphological traits and including all sites, I found that two morphological traits had significant p-values less than 0.05, which included body depth (p-value = 0.01105, t=2.7029) and 3rd Longest raker length (p-value = 0.04085, t=2.1341). In order to visualize one of the distributions of correlation coefficients for a certain trait, I made a histogram of the r_{mantel} values of the 3rd longest raker length and included two scatterplots where one has a very low correlation coefficient (Comida stream had a r_{mantel} value of 0.03233 and a p-value of 0.401) and a high correlation coefficient (Theimer

Stream had a r_{mantel} value of 0.33999 and a p-value of 0.031). It is possible to see in the histogram (**Figure 3)** that the correlation coefficient values are skewed away from zero, even though the majority (26 out of 32) of the mantel results for each site did not have a significant p-value. However, the significant p-value of the t-test shows an overall significant trend of a positive correlation of differences in 3^{rd} longest raker length and spatial distances between traps. This confirms that isolation by distance for this trait occurs within populations, even though the input data comes from traps typically <100m apart.

Table 1. WAIC and pWAIC results for each morphological trait with and without σ_{trap}. Lower WAIC values are shaded and higher pWAIC values are shaded.

Trait	WAIC		pWAIC	
	With σ_{trap}	Without σ_{trap}	With σ_{trap}	Without σ_{trap}
Length 2nd longest raker (mm)	63.74	395.36	230.27	19.62
Length 3rd longest raker (mm)	88.89	378.27	220.74	19.90
Length longest raker (mm)	121.82	6068.78	224.10	19.12
Length 3rd longest raker (mm) (sc)	3020.22	354.92	218.22	19.39
Length 2nd longest raker (mm) (sc)	3019.87	370.86	217.58	20.01
Length longest raker (mm) (sc)	3270.68	393.90	240.51	19.23
Caudal depth (mm) (lateral.sc)	5591.85	1794.91	239.97	19.19
Body width anal 2 (mm) (sc)	3021.84	2384.42	219.95	18.75
Mouth length (mm) (lateral.sc)	2512.66	2653.32	169.19	19.14
Pectoral fin insertion length (mm) (lateral.sc)	2658.81	2891.22	207.04	19.50
Gape width (mm) (sc)	2830.89	3028.85	211.51	19.24
Left side pelvic spine length (mm) (sc)	3013.39	4457.51	215.77	19.50
Mass (g) (sc)	3015.92	4176.54	215.18	20.28
Right side pelvic spine length (mm) (sc)	3016.13	4975.01	216.11	19.56
Raker density per (mm) (sc)	3059.54	3266.03	221.99	20.57
Eye diameter (mm) (lateral.sc)	3083.13	3305.80	196.80	23.29
Body width eye (mm) (sc)	3126.07	3229.26	173.34	19.12
Raker density per (mm)	3181.31	3429.54	215.14	20.51
Snout length (mm) (lateral.sc)	3542.57	3862.98	227.81	19.56
Body width anal 1 (mm) (sc)	4882.40	3590.62	237.00	18.71
Dorsal spine 1 length (mm) (lateral.sc)	3901.79	4411.55	264.14	19.87
Pelvic girdle diamond width (mm) (sc)	3935.33	4186.03	232.75	18.81
Pelvic girdle width (mm) (sc)	4148.04	4428.42	241.37	19.44
Body width midbody (mm) (sc)	4225.96	4406.18	206.17	19.26
Pectoral fin length (mm) (lateral.sc)	4363.98	4650.69	233.53	19.98
Dorsal spine 2 length (mm) (lateral.sc)	5593.83	4527.02	242.63	19.83
Pelvic girdle diamond length (mm) (sc)	4884.48	5192.53	238.72	18.77
Buccal cavity length (mm) (sc)	5295.21	5557.82	217.69	18.85
Anal fin length (mm) (lateral.sc)	5513.10	5830.77	224.52	19.12
Pectoral fin width length (mm) (lateral.sc)	5525.55	5741.62	209.15	19.54
Pelvic girdle length (mm) (sc)	5532.61	5953.24	252.55	19.69
Body depth (mm) (lateral.sc)	5601.33	5930.47	243.35	19.63
Head length (mm) (lateral.sc)	5741.51	6300.34	19.39	20.13
Dorsal fin length (mm) (lateral.sc)	6097.94	6329.53	216.75	19.57
Pectoral fin perimeter (mm) (lateral.sc)	7142.05	7377.48	215.71	20.46
Right side gill raker number insitu	7304.13	7303.82	3.24	3.68
Right side plate count	7313.43	8345.01	211.57	40.14
Left side plate count	8236.88	8805.72	220.63	21.72
Pectoral fin area (mm) (lateral.sc)	74209.04	74165.26	231.60	220.21

Table 2. Means and standard deviations of distances between overall traps and nearest neighboring trap. Shaded sites signify having at least one significant p-value < 0.05 from the Mantel tests.

Site Name	Mean Trap-Trap Distance (m)	St. Dev. Trap-Trap Distance (m)	Mean nearest neighbor (m)	St. Dev. nearest neighbor (m)
beaver.L	71.5420088	48.6377102	6.44095278	5.08149462
beaver.S	38.3585053	27.661597	12.495205	5.51936078
boot.L	31.9495178	26.821758	7.54342134	10.5215611
boot.S	24.7843551	15.2480305	5.47492683	3.55947069
comida.L	39.6209127	26.7923917	3.08365925	2.21344133
comida.S	41.840276	35.3002674	4.70644622	4.13593862
frederick.L	73.3837503	48.734883	9.13848188	6.84321785
frederick.S	158.923018	108.07497	11.5422371	11.7059033
joe.L	44.5128245	32.0542076	3.20230445	2.16028334
joe.S	10.2480261	6.84549421	2.70656448	2.15577932
kennedy.L	34.6152859	24.0366523	8.01445714	6.99248143
kennedy.S	36.1303237	23.7037987	9.75177796	7.8799181
misty.L	37.5373922	22.4295857	8.81084563	6.25697605
misty.S	34.5434507	23.2010425	6.17134044	4.22580509
moore.L	22.3647054	17.4421634	5.9168131	9.30623661
moore.S	11.6943968	7.65727267	2.86009645	1.27990976
muchalat.L	101.234848	64.5856177	15.8456536	14.7130754
muchalat.S	69.4265376	65.1005984	3.69855256	3.15739882
northy.L	13.545566	10.7311163	1.99612885	0.71903865
northy.S	10.8145677	7.2653936	2.7310009	1.33962979
pachena.L	29.7314831	19.0574014	9.35077826	10.5392379
pachena.S	61.1900134	39.8965403	8.70915301	6.22656683
pye.L	1032.40896	795.278931	28.9604532	56.9616531
pye.S	21.5232114	13.2488551	4.32406235	1.43431382
roberts.L	51.5047558	33.6236295	3.57675425	2.78696438
roberts.S	29.5227674	19.2333473	3.14527982	1.55532337
swan.L	14.0439392	8.70400287	3.77141826	1.79828803
swan.S	10.9235514	8.42306137	2.85676617	3.16323547
thiemer.L	34.5329217	23.5280354	4.99815251	5.26631527
thiemer.S	18.8219142	12.0773333	5.89958702	4.56009273
village.bay.L	24.3857911	14.101309	3.64996488	3.47112991
village.bay.S	8.4723165	5.17019611	4.73715099	2.03855577

Table 3. Precis results for each morphological trait

Trait	Mean α	Mean $\alpha_{habitat}$	Mean σ_{trap}	Mean $\sigma_{watershed}$	Mean σ	lower α	lower $\alpha_{habitat}$	lower σ_{trap}	lower $\sigma_{watershed}$	lower σ	upper α	upper $\alpha_{habitat}$	upper σ_{trap}	upper $\sigma_{watershed}$	upper σ
Left side plate count	7.88	1.87	3.3	3.71	3.7	6.25	1.14	3.03	2.52	3.56	9.24	2.45	3.56	4.94	3.83
Right side plate count	5.85	7.98	1.85	3.34	3.75	3.77	-3.02	6.36	1.19	3.09	2.45	3.63	13.77	9.35	2.43
Right side gill raker number insitu	19.07	-0.07	1.29	0.87	1.46	18.69	-0.29	1.18	0.57	1.41	19.41	0.18	1.39	1.16	1.51
Length longest raker (mm)	0.98	0.06	0.17	0.09	0.23	0.93	0.03	0.15	0.06	0.22	1.02	0.1	0.18	0.12	0.24
Length 2nd longest raker (mm)	0.94	0.06	0.17	0.09	0.23	0.9	0.03	0.15	0.06	0.22	0.99	0.09	0.19	0.12	0.23
Length 3rd longest raker (mm)	0.93	0.06	0.16	0.09	0.23	0.88	0.02	0.14	0.06	0.22	0.97	0.09	0.18	0.13	0.24
Raker density per (mm)	3.27	0.04	0.43	0.38	0.66	3.11	-0.05	0.38	0.26	0.64	3.41	0.13	0.47	0.49	0.68
Dorsal spine 1 length (mm) (lateral.sc)	4.46	0.15	0.75	0.74	0.83	4.16	0.02	0.69	0.54	0.8	4.81	0.29	0.81	0.95	0.86
Dorsal spine 2 length (mm) (lateral.sc)	5.26	0.11	0.79	0.73	0.86	4.94	-0.02	0.72	0.52	0.82	5.53	0.26	0.85	0.94	0.89
Dorsal fin length (mm) (lateral.sc)	11.71	0.09	1.14	0.83	1.78	11.38	-0.14	1.02	0.55	1.73	12.08	0.33	1.27	1.09	1.85
Caudal depth (mm) (lateral.sc)	2.03	0.01	0.27	0.2	0.37	1.96	-0.03	0.24	0.14	0.35	2.12	0.07	0.29	0.26	0.38
Anal fin length (mm) (lateral.sc)	8.51	0.02	0.01	0.75	1.76	8.2	-0.14	0	0.53	1.71	8.78	0.18	0.01	0.95	1.82
Pectoral fin insertion length (mm) (lateral.sc)	2.6	2.75	-0.03	0.35	0.26	0.55	1.43	2.66	-0.1	0.31	0.18	0.53	3.48	2.87	0.05
Body depth (mm) (lateral.sc)	10.48	0.13	1.12	0.91	1.48	10.12	-0.07	1.01	0.66	1.44	10.86	0.37	1.22	1.19	1.54
Mouth length (mm) (lateral.sc)	2.37	0.01	0.27	0.17	0.53	2.29	-0.06	0.23	0.1	0.51	2.45	0.07	0.31	0.23	0.55
Snout length (mm) (lateral.sc)	3.55	0.06	0.54	0.34	0.74	3.41	-0.05	0.49	0.21	0.72	3.71	0.16	0.59	0.44	0.77
Eye diameter (mm) (lateral.sc)	4.36	-0.04	0.39	0.27	0.64	4.25	-0.11	0.34	0.18	0.62	4.48	0.05	0.43	0.35	0.66
Head length (mm) (lateral.sc)	13.63	-0.02	1.19	0.82	1.74	13.26	-0.27	1.08	0.56	1.68	13.97	0.2	1.31	1.07	1.8
Pectoral fin width length (mm) (lateral.sc)	7.44	0.13	0.94	0.7	1.49	7.13	-0.07	0.83	0.47	1.43	7.73	0.31	1.04	0.91	1.54
Pectoral fin length (mm) (lateral.sc)	5.89	0.13	0.69	0.56	0.99	5.65	0	0.62	0.39	0.95	6.11	0.28	0.77	0.73	1.02
Pectoral fin perimeter (mm) (lateral.sc)	21.28	0.43	1.68	1.29	2.58	20.69	0.08	1.49	0.93	2.5	21.78	0.83	1.86	1.67	2.68
Pectoral fin area (mm) (lateral.sc)	1.3	-0.16	1.29	15.21	2540207608 0	-12.96	-17.55	0.4	0.53	2461646773 1	25.33	15.53	2.55	41.87	2611400328 1
Buccal cavity length (mm) (sc)	10.34	-0.11	0.91	0.68	1.35	10.07	-0.29	0.82	0.46	1.31	10.68	0.09	1.01	0.88	1.4
Gape width (mm) (sc)	3.07	0.02	0.36	0.29	0.59	2.95	-0.06	0.32	0.2	0.57	3.2	0.1	0.4	0.37	0.61
Body width eye (mm) (sc)	2.6	0.02	0.33	0.26	0.66	2.48	-0.05	0.29	0.16	0.64	2.71	0.1	0.38	0.34	0.68
Body width midbody (mm) (sc)	4.31	0.04	0.57	0.44	0.94	4.12	-0.08	0.51	0.31	0.91	4.5	0.17	0.64	0.57	0.98
Pelvic girdle width (mm) (sc)	4.72	4.86	0.1	0.66	0.57	0.91	3.22	4.65	-0.03	0.6	0.4	0.87	6.42	5.1	0.23
Pelvic girdle diamond width (mm) (sc)	3.16	0.07	0.58	0.5	0.85	2.97	-0.05	0.52	0.35	0.82	3.37	0.18	0.63	0.64	0.88
Pelvic girdle length (mm) (sc)	9.28	0.3	1.2	1.12	1.45	8.87	0.06	1.09	0.79	1.4	9.77	0.5	1.3	1.44	1.5
Pelvic girdle diamond length (mm) (sc)	6.8	0.13	0.86	0.73	1.17	6.52	-0.04	0.78	0.51	1.12	7.11	0.29	0.95	0.94	1.21
Body width anal 1 (mm) (sc)	4.24	0.01	0.5	0.33	0.67	4.1	-0.09	0.46	0.21	0.65	4.4	0.11	0.55	0.43	0.7
Body width anal 2 (mm) (sc)	2.87	2.89	0	0.32	0.24	0.45	2	2.78	-0.06	0.29	0.17	0.44	3.67	2.99	0.07
Mass (g) (sc)	1.72	0.07	0.58	0.46	0.84	1.54	-0.05	0.52	0.32	0.81	1.91	0.19	0.63	0.61	0.87
Left side pelvic spine length (mm) (sc)	6.96	0.27	0.98	0.83	1.03	6.57	0.08	0.9	0.61	0.99	7.28	0.46	1.06	1.07	1.07
Right side pelvic spine length(mm) (sc)	6.95	0.3	1.02	0.91	0.99	6.59	0.12	0.93	0.65	0.96	7.29	0.47	1.09	1.17	1.03
Length longest raker (mm) (sc)	0.98	0.06	0.16	0.09	0.23	0.94	0.03	0.15	0.05	0.22	1.01	0.09	0.18	0.12	0.24
Length 2nd longest raker (mm) (sc)	0.94	0.06	0.17	0.09	0.22	0.91	0.03	0.15	0.06	0.22	0.99	0.09	0.18	0.12	0.23
Length 3rd longest raker (mm) (sc)	0.93	0.06	0.16	0.09	0.23	0.88	0.02	0.14	0.06	0.22	0.97	0.09	0.18	0.12	0.23
Raker density per (mm) (sc)	3.26	0.04	0.41	0.35	0.62	3.11	-0.04	0.37	0.25	0.6	3.4	0.12	0.45	0.45	0.64

Table 4. Mantel test results of Right plate count, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Right Plate Count						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.04706474	0.226	0.775	0.578	0.00766748	0.08616698
beaver.S	0.19386743	0.074	0.927	0.085	0.02084311	0.31277372
boot.L	-0.0919596	0.635	0.366	0.548	-0.1388547	0.01883427
boot.S	0.09539011	0.221	0.78	0.502	-0.1569928	0.23904951
comida.L	0.10102391	0.196	0.805	0.422	-0.0568679	0.32513277
comida.S	0.2108858	0.154	0.847	0.203	0.02860077	0.45413044
frederick.L	0.09581907	0.135	0.866	0.25	0.00546589	0.22183173
frederick.S	0.02478329	0.352	0.649	0.768	-0.1140493	0.08871468
joe.L	0.06643234	0.214	0.787	0.506	-0.0447011	0.21354525
joe.S	0.24721692	0.048	0.953	0.048	0.11933993	0.41566804
kennedy.L	-0.0276919	0.532	0.469	0.848	-0.1585729	0.07932313
kennedy.S	-0.1961833	0.992	0.009	0.098	-0.2593343	-0.1302009
misty.L	-0.2121579	0.942	0.059	0.149	-0.3163916	-0.0562822
misty.S	0.16714648	0.16	0.841	0.272	-0.0227188	0.31352957
moore.L	0.49889929	0.027	0.974	0.027	-0.0355013	0.67995283
moore.S	0.11915635	0.256	0.745	0.572	0.00815425	0.23865974
muchalat.L	0.08047058	0.294	0.707	0.629	-0.1444156	0.36495301
muchalat.S	-0.0846838	0.631	0.37	0.629	-0.1241244	-0.0205667
northy.L	0.00406373	0.388	0.613	0.982	-0.1389205	0.23942956
northy.S	-0.0118053	0.467	0.534	0.946	-0.201117	0.10740731
pachena.L	0.00225802	0.398	0.606	0.997	-0.4335981	0.45956633
pachena.S	-0.0524483	0.627	0.374	0.644	-0.1248451	0.04166264
pye.L	0.21750665	0.014	0.987	0.014	0.08289262	0.38705111
pye.S	0.01150023	0.388	0.613	0.931	-0.0711611	0.12552182
roberts.L	-0.1076407	0.919	0.082	0.19	-0.176861	-0.0240823
roberts.S	-0.0694407	0.758	0.243	0.448	-0.1080556	-0.018099
swan.L	-0.0672815	0.677	0.324	0.571	-0.1712541	0.05613562
swan.S	0.03233161	0.286	0.715	0.873	-0.0934639	0.24158158
thiemer.L	-0.0320723	0.541	0.46	0.749	-0.0966507	0.07288909
thiemer.S	-0.0471661	0.583	0.418	0.707	-0.1335225	0.07077159
village.bay.L	-0.0614941	0.68	0.321	0.585	-0.1240008	0.01307913
village.bay.S	-0.0777697	0.622	0.379	0.615	-0.1997455	0.06400965

Table 5. Mantel test results of Left plate count, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Left Plate Count						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.09093569	0.167	0.834	0.307	0.02340315	0.15979267
beaver.S	0.15932223	0.125	0.876	0.181	0.03367702	0.28677242
boot.L	-0.1511972	0.786	0.215	0.4	-0.2051572	-0.0529318
boot.S	0.45361587	0.003	0.998	0.003	0.26624587	0.67514832
comida.L	0.01876354	0.352	0.649	0.883	-0.0815496	0.1487058
comida.S	0.3024806	0.069	0.932	0.069	-0.0557109	0.53952733
frederick.L	-0.1036516	0.862	0.139	0.281	-0.1552511	-0.0330909
frederick.S	0.01636712	0.389	0.612	0.843	-0.0957101	0.08403633
joe.L	0.1220414	0.131	0.87	0.19	0.0043017	0.26914508
joe.S	0.29410927	0.028	0.973	0.029	0.18731822	0.48086575
kennedy.L	-0.0287111	0.488	0.513	0.857	-0.1744188	0.08871569
kennedy.S	-0.1924263	0.982	0.019	0.12	-0.2743645	-0.1117322
misty.L	0.09679121	0.272	0.729	0.593	-0.025937	0.21023245
misty.S	0.17342886	0.156	0.845	0.194	-0.038873	0.28761286
moore.L	-0.1600742	0.972	0.029	0.141	-0.2063857	-0.1058641
moore.S	0.1447386	0.215	0.786	0.467	-0.0066879	0.27758704
muchalat.L	0.09872644	0.274	0.728	0.601	-0.2362993	0.26596261
muchalat.S	-0.0499473	0.612	0.389	0.704	-0.1487928	0.0347839
northy.L	-0.0613143	0.463	0.538	0.78	-0.222786	0.2065933
northy.S	-0.0848122	0.69	0.311	0.61	-0.1922791	0.03744467
pachena.L	-0.0748648	0.509	0.495	0.863	-0.3061401	0.57436595
pachena.S	-0.0455233	0.606	0.395	0.71	-0.1282909	0.0500986
pye.L	0.074664	0.155	0.846	0.296	-0.0293704	0.22409877
pye.S	-0.1005838	0.773	0.228	0.438	-0.2082714	0.04500542
roberts.L	-0.0705122	0.731	0.27	0.5	-0.1469537	0.04299829
roberts.S	-0.0361637	0.611	0.39	0.715	-0.0999516	0.02697813
swan.L	0.08541916	0.243	0.758	0.517	-0.0954125	0.22075609
swan.S	0.04369012	0.196	0.805	0.767	-0.1792755	0.30738684
thiemer.L	-0.1002503	0.815	0.186	0.376	-0.232759	0.07028218
thiemer.S	0.15121416	0.134	0.867	0.263	0.02981518	0.3122997
village.bay.L	0.00994984	0.368	0.633	0.884	-0.0519758	0.07849914
village.bay.S	-0.1094241	0.733	0.268	0.462	-0.2829332	0.0271244

Table 6. Mantel test results of Pelvic girdle length where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Pelvic Girdle Length (mm) (Lateral)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.06142114	0.188	0.813	0.479	0.00404592	0.1221926
beaver.S	0.15780952	0.145	0.856	0.202	0.05442184	0.2717384
boot.L	-0.1054603	0.636	0.365	0.555	-0.1718192	0.02370522
boot.S	-0.1143775	0.776	0.225	0.418	-0.180681	-0.0382728
comida.L	0.10790557	0.208	0.793	0.438	-0.0600755	0.30677518
comida.S	0.14518623	0.176	0.825	0.34	-0.0985697	0.39743857
frederick.L	0.04026559	0.298	0.703	0.677	-0.0242191	0.11538745
frederick.S	-0.1167787	0.953	0.048	0.127	-0.1461411	-0.0660237
joe.L	-0.0422778	0.61	0.391	0.616	-0.0962381	0.03553439
joe.S	0.03242298	0.354	0.647	0.82	-0.034343	0.13666295
kennedy.L	-0.1172137	0.753	0.248	0.487	-0.1928097	-0.0245228
kennedy.S	0.08719922	0.205	0.796	0.626	-0.0863411	0.29542115
misty.L	0.090835	0.239	0.762	0.493	-0.1405678	0.23535284
misty.S	0.26398063	0.016	0.985	0.016	0.17192008	0.38870511
moore.L	-0.1481571	0.761	0.24	0.514	-0.2067255	-0.0264877
moore.S	0.15226219	0.138	0.863	0.262	0.03464706	0.26328509
muchalat.L	-0.0184212	0.437	0.564	0.903	-0.2765143	0.23458721
muchalat.S	-0.0980482	0.788	0.213	0.399	-0.1402472	-0.048255
northy.L	-0.0445182	0.49	0.511	0.867	-0.1589209	0.1431127
northy.S	-0.1703129	0.906	0.095	0.204	-0.219912	-0.1082723
pachena.L	0.54209823	0.11	0.892	0.11	-0.3662323	0.69462715
pachena.S	-0.0205925	0.545	0.456	0.835	-0.1136133	0.07574335
pye.L	0.08540124	0.151	0.85	0.292	-0.0441046	0.20037364
pye.S	0.14847489	0.154	0.847	0.272	-0.1426737	0.32463723
roberts.L	0.03910749	0.317	0.684	0.741	-0.0191864	0.21182374
roberts.S	-0.0746354	0.847	0.154	0.282	-0.1112437	-0.0305257
swan.L	-0.0994232	0.786	0.215	0.377	-0.1729758	-0.0104141
swan.S	0.11605954	0.191	0.81	0.515	-0.0024304	0.36486274
thiemer.L	-0.1348832	0.973	0.028	0.13	-0.1869988	-0.059325
thiemer.S	0.51991511	0.004	0.997	0.004	0.32247583	0.71640476
village.bay.L	0.12053954	0.129	0.872	0.241	-0.066159	0.21561869
village.bay.S	-0.1598136	0.865	0.136	0.315	-0.2835288	-0.0536074

Table 7. Mantel test results of Head length, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Head Length (mm) (Lateral)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.04723351	0.241	0.76	0.575	-0.0248544	0.15439501
beaver.S	-0.043417	0.462	0.539	0.717	-0.1859756	0.03533792
boot.L	0.17087745	0.14	0.861	0.226	0.0684008	0.5299486
boot.S	-0.1074114	0.76	0.241	0.45	-0.1694363	0.00311011
comida.L	0.29211651	0.027	0.974	0.027	0.15312499	0.43002076
comida.S	0.10226633	0.244	0.757	0.591	-0.1670813	0.30031333
frederick.L	0.11222219	0.137	0.864	0.276	0.03051826	0.23457033
frederick.S	0.14699672	0.032	0.969	0.037	0.0286539	0.2798493
joe.L	-0.074769	0.853	0.148	0.304	-0.1332218	0.0107469
joe.S	0.07892694	0.254	0.747	0.689	-0.001009	0.2509868
kennedy.L	0.60124605	0.001	1	0.001	0.4485678	0.71474615
kennedy.S	0.05319862	0.361	0.64	0.767	-0.0771748	0.31066234
misty.L	-0.0149263	0.465	0.536	0.925	-0.1281686	0.12385396
misty.S	0.1995213	0.035	0.966	0.04	0.11324496	0.31529028
moore.L	-0.1418839	0.839	0.162	0.356	-0.1959105	-0.0684238
moore.S	0.15801403	0.188	0.813	0.333	0.03095514	0.30204415
muchalat.L	-0.0741391	0.506	0.495	0.841	-0.2133617	0.21692183
muchalat.S	0.05110011	0.273	0.728	0.707	-0.0728048	0.2098623
northy.L	-0.1493205	0.805	0.196	0.37	-0.2390058	-0.0363814
northy.S	-0.1122882	0.797	0.204	0.38	-0.2211877	0.0194185
pachena.L	0.60683745	0.049	0.953	0.049	0.17397188	0.90312657
pachena.S	0.15267134	0.065	0.936	0.085	0.01550301	0.28423927
pye.L	0.21861944	0.02	0.981	0.02	0.10489049	0.37104738
pye.S	0.1916759	0.114	0.887	0.179	-0.0854452	0.39248708
roberts.L	0.03264078	0.304	0.697	0.655	-0.0263426	0.12460165
roberts.S	-0.0950571	0.888	0.113	0.247	-0.1314507	-0.0492782
swan.L	0.02953916	0.35	0.651	0.798	-0.0936804	0.1648409
swan.S	0.01431886	0.269	0.732	0.939	-0.1023752	0.17831817
thiemer.L	-0.1283222	0.941	0.06	0.198	-0.191699	-0.0541805
thiemer.S	0.53250603	0.004	0.997	0.004	0.36092856	0.70185534
village.bay.L	0.18512347	0.029	0.972	0.031	0.06141778	0.34723633
village.bay.S	-0.0363405	0.576	0.425	0.754	-0.1686067	0.09341832

Table 8. Mantel test results of Dorsal spine length (2), where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Dorsal Spine Length 2 (mm) (Lateral)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.02670428	0.315	0.686	0.782	-0.0271253	0.11689724
beaver.S	0.08946752	0.138	0.863	0.41	0.02387412	0.2780959
boot.L	-0.0394104	0.469	0.532	0.807	-0.1261565	0.1520524
boot.S	-0.1166597	0.801	0.2	0.404	-0.2311167	-0.0327646
comida.L	0.20227255	0.081	0.92	0.119	0.03895087	0.3850795
comida.S	0.18539849	0.156	0.845	0.21	-0.0781392	0.42439078
frederick.L	-0.0194923	0.55	0.451	0.86	-0.0918851	0.06210016
frederick.S	-0.0395139	0.637	0.364	0.597	-0.1197116	0.01338792
joe.L	-0.0746648	0.859	0.142	0.301	-0.117842	-0.0161286
joe.S	0.21325813	0.11	0.891	0.186	-0.1405174	0.44179211
kennedy.L	-0.0288981	0.558	0.443	0.826	-0.1594452	0.07736571
kennedy.S	0.14564282	0.165	0.836	0.419	0.02090876	0.41625515
misty.L	-0.1459044	0.707	0.294	0.499	-0.2217728	0.01156117
misty.S	0.15589163	0.099	0.902	0.178	0.05685432	0.27482765
moore.L	-0.1589277	0.903	0.098	0.248	-0.2249561	-0.0704275
moore.S	0.22305868	0.116	0.885	0.191	0.1073853	0.36014548
muchalat.L	-0.0554073	0.546	0.455	0.854	-0.2233725	0.13188621
muchalat.S	-0.0714089	0.553	0.448	0.656	-0.1128436	-0.0191309
northy.L	-0.1390885	0.732	0.269	0.514	-0.2258979	0.03108042
northy.S	0.11457008	0.201	0.8	0.423	-0.0099329	0.22542036
pachena.L	-0.38303	0.818	0.183	0.358	-0.6274931	-0.2002098
pachena.S	-0.0704432	0.814	0.187	0.351	-0.1297158	0.00421126
pye.L	0.25323154	0.005	0.996	0.005	0.16845068	0.38441116
pye.S	0.16534924	0.12	0.881	0.212	-0.0064771	0.33197659
roberts.L	0.08457753	0.158	0.843	0.337	0.02310113	0.22747757
roberts.S	0.01130128	0.406	0.595	0.88	-0.0469961	0.07810873
swan.L	-0.1387508	0.909	0.092	0.209	-0.1912677	-0.0640094
swan.S	-0.0473583	0.457	0.544	0.756	-0.1825993	0.14634268
thiemer.L	-0.0861484	0.792	0.209	0.395	-0.1255404	-0.0320704
thiemer.S	0.38992885	0.016	0.985	0.016	0.21695609	0.58974662
village.bay.L	-0.0331437	0.537	0.464	0.801	-0.0942493	0.05233881
village.bay.S	-0.0714851	0.635	0.366	0.619	-0.1887704	0.09556151

Table 9. Mantel test results of Body depth, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Body Depth (mm) (Lateral)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.09367081	0.157	0.844	0.271	0.01197747	0.18598516
beaver.S	-0.0359822	0.48	0.521	0.735	-0.0977328	0.04469928
boot.L	0.05504224	0.264	0.737	0.774	-0.0255666	0.21070191
boot.S	-0.142708	0.882	0.119	0.237	-0.2055789	-0.0384884
comida.L	0.24892697	0.045	0.956	0.053	0.10606596	0.40350144
comida.S	0.12944625	0.263	0.738	0.525	-0.1924857	0.34445022
frederick.L	0.12027707	0.131	0.87	0.224	0.04491697	0.20363881
frederick.S	-0.072251	0.817	0.184	0.338	-0.1284165	0.00731243
joe.L	-0.0634965	0.776	0.225	0.401	-0.1150317	0.00784532
joe.S	-0.0005812	0.43	0.571	0.998	-0.0658578	0.08018862
kennedy.L	0.52526297	0.002	0.999	0.002	0.32247589	0.67576553
kennedy.S	0.10577499	0.234	0.767	0.614	-0.0343456	0.30654288
misty.L	0.04563355	0.282	0.719	0.647	-0.0542342	0.15651487
misty.S	0.25506403	0.022	0.979	0.023	0.15680661	0.37429003
moore.L	-0.1361099	0.766	0.235	0.469	-0.1902234	-0.0080851
moore.S	0.17629906	0.12	0.881	0.225	0.04434427	0.2965632
muchalat.L	0.15186699	0.173	0.828	0.379	-0.1250347	0.4828464
muchalat.S	0.07688561	0.239	0.762	0.506	-0.0573186	0.20406394
northy.L	-0.1552763	0.858	0.143	0.302	-0.2406605	-0.045792
northy.S	-0.1441549	0.804	0.197	0.392	-0.2214048	-0.0136548
pachena.L	0.49783796	0.132	0.874	0.148	-0.3475196	0.7471039
pachena.S	0.12634576	0.119	0.882	0.221	-0.0220928	0.2365317
pye.L	0.26327114	0.01	0.991	0.01	0.1489531	0.38920925
pye.S	0.17263052	0.144	0.857	0.235	-0.0953378	0.36496426
roberts.L	0.02621244	0.358	0.643	0.807	-0.0453901	0.19857216
roberts.S	-0.0841118	0.876	0.125	0.245	-0.1189732	-0.0345423
swan.L	0.0613769	0.233	0.768	0.56	-0.0536798	0.15447326
swan.S	0.17478409	0.11	0.891	0.188	0.03781163	0.40272794
thiemer.L	-0.1582035	0.979	0.022	0.114	-0.232993	-0.0614508
thiemer.S	0.54165542	0.003	0.998	0.003	0.37759533	0.72240962
village.bay.L	0.14498131	0.086	0.915	0.113	-0.0562611	0.24244675
village.bay.S	-0.1088883	0.765	0.236	0.462	-0.2205831	0.05135742

Table 10. Mantel test results of Right side gill raker number, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of Right Side Gill Raker Number (insitu)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	0.11636784	0.125	0.876	0.194	-0.03772	0.19794897
beaver.S	-0.0364509	0.586	0.415	0.74	-0.1167307	0.10536506
boot.L	-0.143488	0.885	0.116	0.312	-0.1903438	-0.0795105
boot.S	-0.1414874	0.861	0.14	0.294	-0.2039731	-0.0668453
comida.L	0.25081582	0.05	0.951	0.051	0.10846466	0.44928451
comida.S	0.00775037	0.323	0.678	0.952	-0.0899985	0.1804741
frederick.L	-0.171762	0.962	0.039	0.099	-0.2381536	-0.1060911
frederick.S	-0.0102902	0.522	0.479	0.891	-0.0695739	0.0767068
joe.L	0.08564805	0.178	0.823	0.391	-0.0214639	0.20147443
joe.S	0.33967711	0.028	0.973	0.028	0.2140552	0.53878908
kennedy.L	-0.2054786	0.993	0.008	0.129	-0.2614274	-0.1526733
kennedy.S	-0.0617124	0.598	0.403	0.714	-0.1877169	0.10876082
misty.L	0.19630097	0.16	0.841	0.271	0.0892503	0.41923692
misty.S	-0.0371438	0.556	0.445	0.801	-0.1961284	0.10477533
moore.L	0.04541201	0.275	0.726	0.821	-0.1873358	0.31164234
moore.S	0.29357976	0.097	0.904	0.097	0.03177199	0.45949555
muchalat.L	0.19041323	0.238	0.763	0.518	-0.1302951	0.71764583
muchalat.S	-0.028565	0.525	0.476	0.825	-0.1492686	0.07351331
northy.L	-0.1208877	0.647	0.354	0.559	-0.2096228	-0.0074123
northy.S	0.04966977	0.337	0.664	0.711	-0.0144901	0.14785135
pachena.L	0.15092542	0.281	0.723	0.672	-0.3784248	0.52906401
pachena.S	-0.0014247	0.45	0.551	0.985	-0.0650042	0.06465004
pye.L	0.08865015	0.116	0.885	0.205	-0.0053476	0.26307453
pye.S	-0.1229084	0.791	0.21	0.375	-0.1961583	0.06955833
roberts.L	-0.0820428	0.852	0.149	0.301	-0.1363187	0.00062807
roberts.S	0.07428065	0.16	0.841	0.298	0.01487817	0.1415575
swan.L	-0.0591323	0.633	0.368	0.692	-0.113693	0.01344667
swan.S	0.09949667	0.216	0.785	0.617	-0.0306999	0.2600654
thiemer.L	-0.1211616	0.9	0.101	0.221	-0.2232351	-0.0117588
thiemer.S	-0.1038019	0.772	0.229	0.453	-0.1870617	0.03177386
village.bay.L	-0.0212012	0.51	0.491	0.861	-0.1271825	0.07470101
village.bay.S	-0.2166562	0.986	0.015	0.073	-0.2917916	-0.1262105

Table 11. Mantel test results of 3rd longest raker length, where shaded sites contained a significant p-value < 0.05.

Mantel Test results of 3rd Longest Raker Length (mm)						
Site Name	mantelr	pval1	pval2	pval3	llim.2.5%	ulim.97.5%
beaver.L	-0.0299728	0.554	0.447	0.764	-0.0923435	0.04237502
beaver.S	-0.1766084	0.98	0.021	0.134	-0.2248601	-0.1245764
boot.L	0.25349753	0.124	0.877	0.124	0.1268699	0.50438643
boot.S	-0.061615	0.632	0.369	0.638	-0.1404157	0.10581545
comida.L	0.19596675	0.09	0.911	0.156	0.01439282	0.36750679
comida.S	0.03233584	0.399	0.602	0.863	-0.137022	0.1495631
frederick.L	0.05163583	0.286	0.715	0.587	-0.0249194	0.13588588
frederick.S	0.17988399	0.027	0.974	0.027	0.0826322	0.34108908
joe.L	-0.0933965	0.939	0.062	0.173	-0.1457694	-0.0250377
joe.S	0.16898511	0.141	0.86	0.278	0.03146638	0.29032312
kennedy.L	0.09976778	0.262	0.739	0.622	-0.1761155	0.29310642
kennedy.S	-0.0804048	0.654	0.347	0.619	-0.3249343	0.24020794
misty.L	0.34076397	0.053	0.948	0.053	0.22856701	0.53082535
misty.S	0.14801591	0.095	0.906	0.185	0.03277001	0.2805015
moore.L	-0.161541	0.783	0.218	0.438	-0.2325002	-0.0565075
moore.S	0.14435543	0.176	0.825	0.369	0.03255666	0.26776354
muchalat.L	-0.1303756	0.756	0.245	0.447	-0.2605776	0.03597516
muchalat.S	0.32398455	0.036	0.965	0.036	0.14630869	0.46065256
northy.L	-0.1454476	0.869	0.132	0.297	-0.2522592	-0.0352148
northy.S	0.12837915	0.188	0.813	0.355	-0.0225414	0.29347874
pachena.L	0.64619368	0.033	0.968	0.033	0.41842077	0.85642022
pachena.S	0.20843564	0.041	0.96	0.043	0.04346435	0.34865632
pye.L	0.00215089	0.398	0.603	0.981	-0.0714824	0.07028104
pye.S	0.08312799	0.241	0.76	0.527	-0.0694188	0.23402428
roberts.L	0.08091513	0.221	0.78	0.434	-0.0016475	0.19619892
roberts.S	-0.0979901	0.884	0.117	0.235	-0.1394368	-0.0520254
swan.L	-0.1735351	0.969	0.032	0.115	-0.262183	-0.1026165
swan.S	-0.0154027	0.371	0.63	0.935	-0.1437299	0.12204252
thiemer.L	0.03973709	0.248	0.753	0.67	-0.0582848	0.21479481
thiemer.S	0.33999759	0.019	0.982	0.019	0.20045036	0.50406358
village.bay.L	-0.0120023	0.521	0.48	0.914	-0.0944166	0.08173114
village.bay.S	-0.0639068	0.67	0.331	0.635	-0.1477621	0.0635378

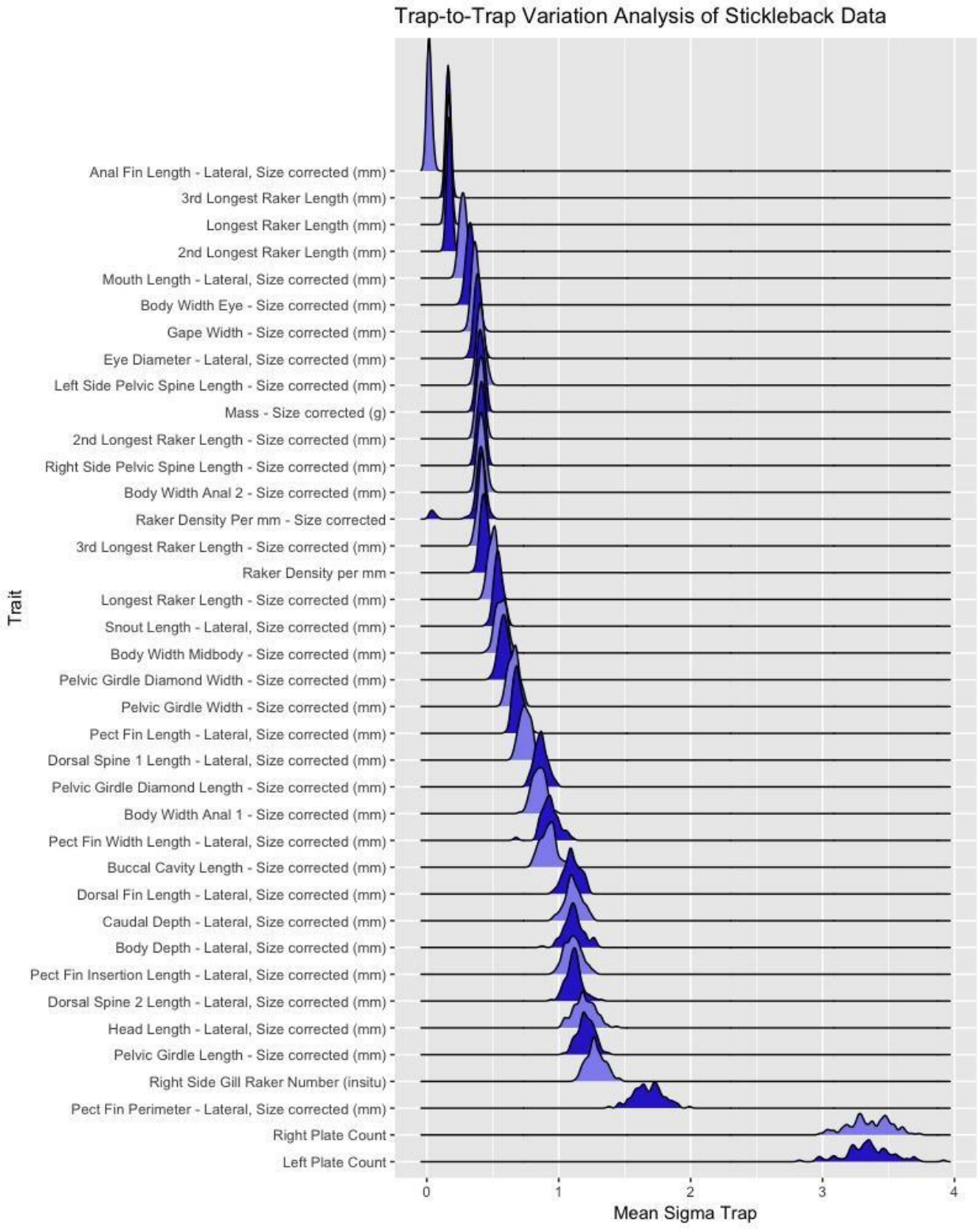


Figure 2. Posterior probability densities of the mean σ_{trap} for each morphology.

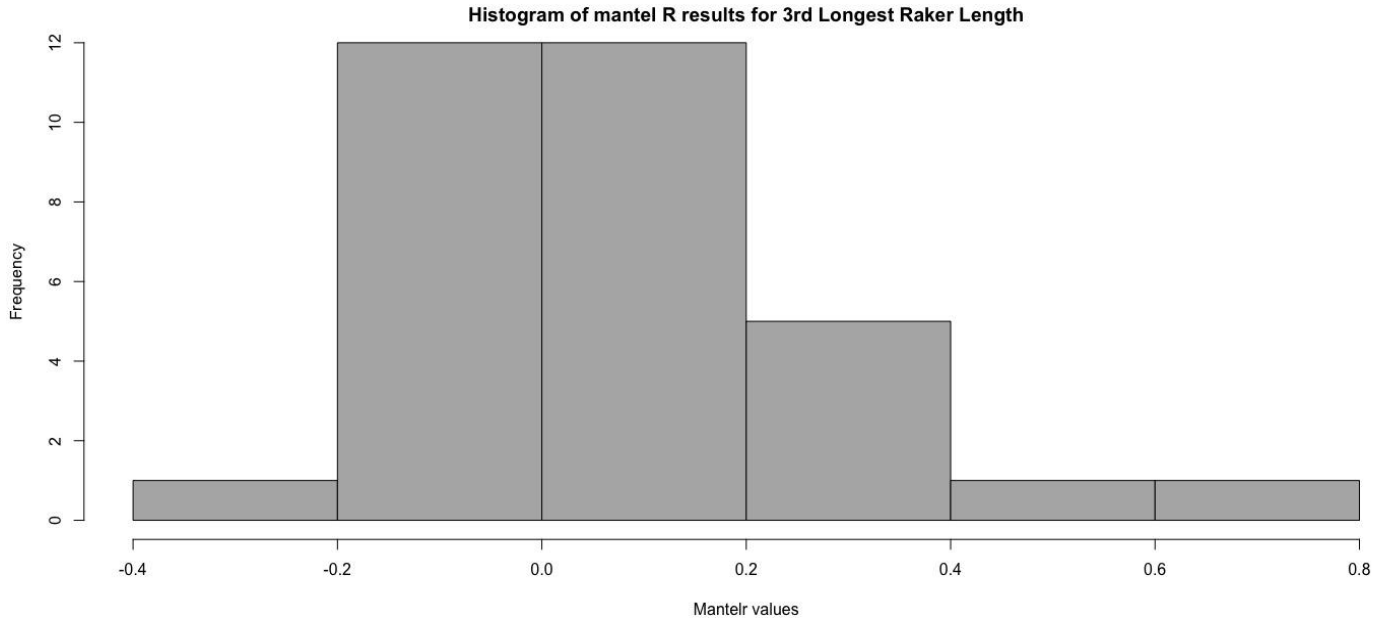


Figure 3. Histogram of the distribution of Mantelr correlation coefficient values of 3rd longest raker length.

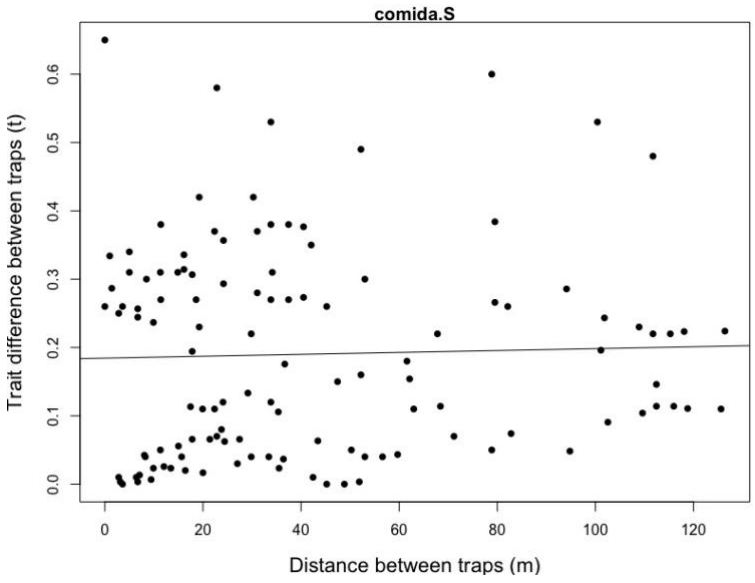


Figure 4. Scatterplot of trait difference and trap distance of Comida Stream, with correlation coefficient of 0.03233

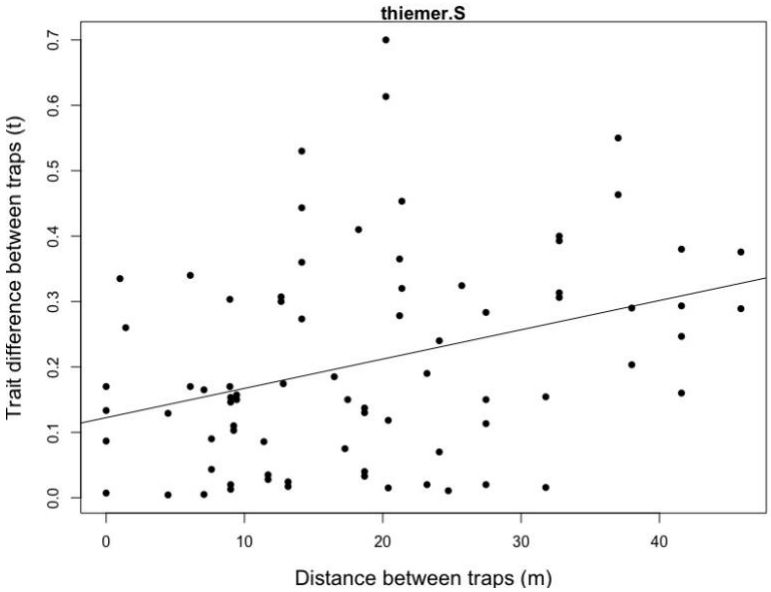


Figure 5. Scatterplot of trait difference and trap distance of Thiemer Stream, with correlation coefficient of 0.33999.

Discussion

Our analysis demonstrated that stickleback morphology differs from trap to trap within lakes, and within streams, and therefore provides support for microgeographic divergence. Despite the traps being placed within meters from their nearest neighbor, there was still spatial variation in morphology of stickleback. This is interesting because it is known that the dispersal neighborhood of sticklebacks is about 40 meters movement (median) over 4 days (Bolnick et al., 2009). My analysis also demonstrates that there is an overall significant correlation between spatial and phenotypic difference in two of the morphological traits of stickleback among all the habitats I looked at, including 3rd longest raker length and body depth. In addition, when looking between habitats, certain sites had significant correlation between spatial and phenotypic difference as well but the majority of the sites do not exhibit significant phenotypic differences due to spatial isolation in the eight morphological traits I looked at. We explore other possible reasons facilitating this variation, including spatial isolation, assortative schooling, microhabitat choice, and phenotypic plasticity.

The traits that had the most among-trap variation was lateral plate count (left and right), pectoral fin perimeter, right side gill raker number, and pelvic girdle length. Research looking at variation in lateral plate size and count attribute possible differences due to other factors such as shifts in swimming and buoyancy, as well as having limited amount of minerals to bone development, and allocating energy to reproduction or growth as opposed to plate growth (Wiig et al., 2016). The pectoral fin perimeter is an indication of the shape of the pectoral fin, a trait that helps to determine the propulsion and maneuverability of a fish (Walker, 2016). The

geometry of the fin can also play a large role in how the stickleback interacts with its environment and its resources.

The right side gill raker number gives us insight into the differences in feeding behaviors between stickleback, since gill raker number often determines the feeding habits of the fish, and aggregation may be the result of differences in food availability and size between traps (Glazer et al., 2014). Finally, pelvic girdle may also play a role in maneuverability of the fish similarly to the pectoral fin perimeter, allowing for greater adaptability in certain flow rates or depths in the water column. Robust pelvic girdles are also found to be maintained in stickleback populations as a form of defense against predators (Lescak & von Hippel, 2011).

Each individual trait varies in the number of habitats that experience significant positive correlations between trait and spatial distance between the traps (variation due to spatial isolation). For example, mantel tests on the right side gill raker number (insitu) show only one site having a significant positive correlation, whereas mantel tests on the head length show a total of nine sites having a significant positive correlation. After performing t-tests, I do not find an overall positive correlation in all the habitats as a whole for any trait besides 3rd longest raker length and body depth (out of the eight I looked at). Therefore, I can conclude that for traits besides 3rd longest raker length and body depth, there is not an overall significant trend towards a positive correlation of phenotypic and spatial differences. However, there still exists significant positive correlation within some individual sites.

The varying numbers of sites that exhibit spatial isolation can give more insight into the role of individual morphological traits and the individual sites as well. Since Pye Lake demonstrated clustering of traps, it was not surprising that it also has the highest number of significant p-values and therefore has the most spatial isolation. The same can be seen in the

distribution of traps within Comida Lake which also had the highest number of significant p-values demonstrating spatial isolation. However, it is interesting that despite its clustering, these two sites did not have mantel test results with significant positive correlations in all of the eight traits and could mean that spatial isolation is not the only mechanism facilitating these positive correlations for these habitats. Other mechanisms, such as assortative schooling, microhabitat choice, and phenotypic plasticity, could be facilitating these variations as well.

Assortative schooling refers to the self-referent matching behavior exhibited in social animals with regard to phenotype and preferentially matching with conspecifics (Ward, Hart & Krause, 2004). Research has shown that stickleback have developed preferences for unrelated familiar individuals (Utne-Palm and Hart, 2000), and therefore stickleback with similar morphologies can aggregate in traps and be the reason for similar fish to be found in the same traps. Phenotypic plasticity has already been shown to be a driving mechanism for a depth gradient in male conspicuousness in stickleback (Brock, Cummings & Bolnick, 2017), and could also explain differences in morphologies between traps within a habitat. These stickleback could possibly randomly choose their habitat, and their morphology can change over time in order to adapt to their respective habitat, creating microhabitats with stickleback of similar morphologies. Research has also shown that stickleback can choose microhabitats (Brock, Cummings & Bolnick, 2016; Bolnick et al., 2009) and that their performance in these habitats depend on their traits, therefore possibly explain aggregations of fish with similar morphological traits within traps.

The importance of this research is that it reinforces that microgeographic divergence, which is often thought to be rare, can more widespread than we think despite the effects of gene flow. Local adaptation is explored intensively in large spatial scales, and the increasing amount

of research and evidence of microgeographic adaptation makes us rethink the spatial scale at which evolutionary adaptations occur.

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